RECEPTOR AND TRANSPORTER ANTAGONISTS

Related Application

This application claims priority from U.S. Provisional Patent Application No. 60/014,306 filed on March 27, 1996.

Field of the Invention

This invention relates to the field of integral membrane proteins which act as receptors or signal transducers. More specifically, it relates to the identification and preparation of specific antagonists of the function of such proteins.

Background of the Invention

Various journal articles referred to herein are identified by authors and date in parentheses and are listed, with full citations, at the end of the specification.

Receptors are the primary targets and mediators of hormone and drug actions. The cell surface receptors, such as the G protein-coupled receptors (GPCRs), ion channel receptors and tyrosine kinase receptors, belong to gene superfamilies based on sequence and structural similarities. Receptors belonging to these superfamilies are all integral membrane proteins predicted to exhibit extracellular, hydrophobic membrane-spanning and intracellular domains. Whereas tyrosine kinase receptors exhibit a single membrane spanning domain, G protein-coupled receptors are defined by seven putative hydrophobic membrane spanning segments which have become the hallmark of this gene superfamily. The biogenic amine transporter proteins are also membrane-spanning proteins with twelve transmembrane segments that mediate the reuptake of released neurotransmitter. The ion channel receptors generally have separate subunits that associate together to form a functional receptor.

The amino acid sequence of a receptor protein, which is unique to each receptor, confers specific structure-related functions to the receptor, while conforming to the general structural determinants of the particular class of protein to which it belongs. The amino acid sequence of an integral membrane protein, such as a receptor or transporter protein, determines the hydrophobic and hydrophilic portions, and has been used in the development of algorithms for the prediction of membrane protein secondary structure (Engelman et al., 1986, Kyte and Doolittle, 1982). Peptide probes derived from regions of various proteins have been used for mapping structural determinants of proteins. This has been best characterized for the single transmembrane spanning sialoglycoprotein, glycophorin A, isolated from human erythrocyte membranes (Furthmayr and Marchesi, 1976). In these studies, a small hydrophobic peptide derived from glycophorin A was able to prevent the association of 2 subunits in vitro, which suggested that native glycophorin A in membranes is composed of subunits associated with each other by hydrophobic portions of the polypeptide chains. Subsequent studies demonstrated that a specific molecular motif (LIxxGVxxGVxxT) participates in the formation of glycophorin A dimers (Bormann et al., 1989, Lemmon et al., 1992).

For the G protein coupled receptors, Okamoto et al. (1991) used a synthetic peptide corresponding to the end of the third cytoplasmic loop of the G protein-coupled β2-adrenergic receptor to demonstrate that this amino acid sequence was critical for this receptor's ability to activate G proteins. In addition, it has been shown that coexpression of the third intracellular domain of the G protein-coupled α1B-adrenergic receptor along with the receptor specifically inhibited receptor-mediated inositol phosphate production (Luttrell et al., 1993),

and that expression of the intracellular third loop of the dopamine D1 receptor specifically inhibited receptor-mediated cAMP production (Hawes et al., 1994).

GPCRs have been shown to exist as dimers and monomers in the presence of denaturants and reducing agents (Ng et al., 1993, 1994a, 1994b), suggesting that dimerisation does not occur via covalent disulphide bonds. It has been hypothesised that the seven transmembrane(TM) spanning domains of GPCRs are sequentially arranged in an anti-clockwise circular manner forming a TM receptor core (Baldwin, 1993). The proper folding of GPCR monomers is suggested to involve intramolecular interactions between TMI and TMVII (Kobilka et al., 1988, Suryanarayana et al., 1992). Coexpression studies with chimeric GPCRs have suggested that receptor interactions may involve TMVI and VII (Maggio et al., 1993, Liu et al., 1995), and mutagenesis studies have demonstrated that antagonist interaction with the receptors may involve these TM regions.

The importance of membrane-spanning proteins such as receptors and transporters in signal transmission across the cell membrane makes these proteins prime targets for pharmaceutical intervention in many disorders. There are presently few highly selective pharmaceuticals available for treatment of such disorders. There is therefore a great need for drugs useful for the prevention or treatment of receptor-mediated disorders, and for the selective targeting of receptor/transporter functions, to aid in the treatment of commonly occurring and highly prevalent diseases.

Brief Description of the Drawings

The invention, as exemplified by preferred embodiments, is described with reference to the accompanying drawings in which:

Figure 1 shows a two dimensional representation of the seven membrane-spanning domains of the D2 dopamine receptor.

Figures 2A to 2G show immunoblots of isolated dopamine receptors after various treatments, monomers (M) and dimers (D) being indicated by arrows.

Figure 2A shows the effect of the indicated concentrations of D2-TM VI peptide (aa 375-394) on the electrophoretic pattern of the D2 receptor (Lanes 1-4: 0, 1.3, 1.6 and 2.5 $\mu g/\mu l$ D2-TM VI peptide, respectively).

Figure 2B shows the effect of the indicated concentrations of D2-TM VII peptide (aa407-426) on the D2 receptor (Lanes 1-7: 0, 0.3, 0.6, 1.0, 1.3, 1.6 and 2.5 $\mu g/\mu l$ D2-TM VII peptide).

Figure 2C shows the effect of incubation with the D2-TM VI and D2-TM VII peptides on the D2 receptor from human caudate nucleus. Lanes 1 and 3: buffer control; lane 2: D2-TM VI peptide; lane 4: D2-TM VII peptide.

Figure 2D shows the effect of hydrophilic and hydrophobic receptor peptides on a D2 receptor preparation. D2 receptors were incubated in peptide buffer (lane 1); D2-C IIIA peptide (aa 244-263) (lane 2); D2-C IIIB peptide (aa 284-303) (lane 3); β2-AR TM VI peptide (aa 276-296) (lane 4); D1-C IIIA peptide (aa 369-383) (lane 5); and D1-C IIIB peptide (aa 416-431) (lane 6).

Figure 2E shows the effect of D2-TM VII peptide on c-myc epitope-tagged human dopamine D1 receptor and c-myc epitope-tagged human serotonin 5-HT1B receptor. D1 receptors were incubated in peptide buffer without (lane 1) or with (lane 2) D2-TM VII peptide, and 5-HT1B receptors were incubated in peptide buffer without (lane 3) or with (lane 4) D2-TM VII peptide.

Figure 2F shows the effect of temperature on the D2 dopamine receptor.

Figure 2G shows the effect of pH on the D2 dopamine receptor (GAA = glacial acetic acid).

Figure 3 shows the duration (X axis) and extent (Y axis) of asymmetric body response of a rat after unilateral (left) intrastriatal injection of D2-TM VII peptide, 15 ng/3 μl (Figure 3A), peptide vehicle (Figure 3B), and β2-AR VI peptide, 15 ng/3 μl (Figure 3C).

Figure 4 shows the duration (X axis) and extent (Y axis) of asymmetric body response of a rat with bilateral cannulae after left unilateral intrastriatal injection of D2-TM VII peptide, 15 ng/3 μl (vehicle injection into the right contralateral striatum).

Figures 5a to 5h show polygraph traces of blood pressure (Y axis: mm Hg) in a rat at indicated time intervals (X axis) after treatment with various agents:

Figure 5a : baseline;
Figure 5b : 1 μg/Kg isoproterenol;
Figure 5c : 500 μg LYSAFTWLGYUNSAVNPIIY (B1-TM7);
Figure 5d : 1 μg/Kg isoproterenol.
Figure 5e : baseline;
Figure 5f : 1 μg/Kg isoproterenol;
Figure 5g : vehicle;
Figure 5h : 1 μg/Kg isoproterenol.

Figures 6a to 6g show polygraph traces of blood pressure (Y axis: mm Hg) in a rat at indicated time intervals (X axis) after treatment with various agents:

Figure 6a : baseline;
Figure 6b : 5 μg/Kg phenylephrine;
Figure 6c : 500 μg FFNWLGYANSAFNP (alpha TM7);
Figure 6d : 5 μg/Kg phenylephrine.
Figure 6e : saline;
Figure 6f : 5 μg/Kg phenylephrine;
Figure 6g : vehicle.

Figures 7a to 7d show polygraph traces of blood pressure (Y axis: mm Hg) in a rat at indicated time intervals (X axis) after treatment with various agents; Figure 7a: baseline; Figure 7b: 5 μg/Kg phenylephrine; Figure 7c: 1 mg/Kg prasozin; and Figure 7d: 5 μg/Kg phenylephrine.

Detailed Description of the Invention

The inventors have shown that a peptide which has the amino acid sequence of a hydrophobic or transmembrane domain of an integral membrane protein, or of a portion of the transmembrane domain, has a specific and selective antagonistic effect on the activity or function of the integral membrane protein from which it is derived. This antagonistic effect has been shown both *in vitro* and *in vivo*, in animal models.

It is hypothesised that the mechanism of the antagonism exerted by transmembrane domain peptides is the binding of such peptides to a transmembrane domain of the integral membrane protein, thereby interfering with intramolecular interactions which contribute to the proper three-dimensional conformation of the integral membrane protein monomer. The formation of a heterodimer of the antagonist peptide and the integral membrane protein monomer will interfere with binding of the integral membrane protein with its ligand and, for integral membrane proteins which are normally associated as dimers, will interfere with dimer formation.

It is predicted that all integral membrane proteins having one or more transmembrane domains will be susceptible to disruption of their function by a peptide having the amino acid sequence of any one of their own transmembrane domains. An antagonist peptide having a transmembrane amino acid sequence of a particular integral membrane protein shows specificity for that

protein and does not interfere with the function of closely related integral membrane proteins.

Integral membrane proteins comprise a great variety of proteins, including signal transducing proteins such as G-protein coupled receptors and tyrosine kinase receptors, transporter proteins, membrane channel proteins, T cell receptors and adhesins.

Integral membrane proteins are found in the cell membranes of prokaryotic and eukaryotic cells and also within intracellular membranes in eukaryotic cells, for example the endoplasmic reticulum intracellular transporter, lysosomal membrane proteins and sialoglycoproteins. Antagonist peptides in accordance with the present invention can be used to control the function of integral membrane proteins found in all these various locations.

Also included within the scope of the invention are fragments or analogues of the transmembrane amino acid sequences of an integral membrane protein which are effective to antagonise the function of that protein. A fragment or analogue of a transmembrane amino acid sequence of an integral membrane protein is effective if it is a functional equivalent of the transmembrane amino acid sequence.

The transmembrane or membrane-spanning domains of integral membrane proteins are believed to have a helical conformation and generally comprise a sequence of about 22 to 26 amino acids.

The antagonist peptide for a particular integral membrane protein may have the entire amino acid sequence of a transmembrane domain or may comprise a portion or fragment of the transmembrane amino acid sequence.

Fragments of a transmembrane amino acid sequence may be selected by truncation of one or more amino acids from the amino terminus of the transmembrane amino acid

sequence, by truncation of one or more amino acids from the carboxy terminus or by truncation of one or more amino acids from both amino and carboxy termini.

As is understood by those skilled in the art, in the identification of a transmembrane amino acid sequence within the total amino acid sequence of an integral membrane protein, there may be a variation of one or two amino acids in defining the termini of the transmembrane amino acid sequence, depending on the hydropathy analysis software used.

This possible variation does not limit the ability of one skilled in the art to select antagonist peptides or fragments in accordance with the invention.

The present invention provides antagonist peptides which correspond to the amino acid sequence of an integral membrane protein transmembrane domain, fragments of such a sequence and peptides which include the amino acid sequence of an integral membrane protein transmembrane domain or fragments thereof.

The present invention provides antagonist peptides comprising amino acid sequences corresponding to at least four, preferably ten and more preferably from fifteen to twenty consecutive amino acids of an integral membrane protein transmembrane domain.

The amino acid sequences of the transmembrane domains of integral membrane proteins are highly conserved in mammals.

The function of an integral membrane protein from a first species may be antagonised by a peptide corresponding to the amino acid sequence of one of its own transmembrane domains or may be antagonised by a functionally equivalent transmembrane domain amino acid sequence from the corresponding region of the integral membrane protein of a second species. The term "functionally equivalent" means that the sequence of the

transmembrane domain of the second species need not be identical to that of the first species but need only comprise a sequence which functions biologically and/or chemically as the equivalent of the transmembrane amino acid sequence of the first species.

The present invention provides a generally applicable means of selecting a suitable specific antagonist for inhibition or reduction of the activity of a target integral membrane protein such as a receptor or transporter.

In addition to the antagonist peptides disclosed herein, one of ordinary skill in the art is now enabled to identify and prepare antagonist peptides specific for any selected integral membrane protein. For example, a number of integral membrane proteins of different types are listed in Table 3 with their Accession Numbers in the SwissProt database. One of ordinary skill in the art can obtain, through such accession numbers, the amino acid sequence of a selected integral membrane protein, with identification of its transmembrane domains.

Using the present disclosure and integral membrane protein amino acid sequence information available either in the scientific literature or in databases such as GenBank or SwissProt, one of ordinary skill in the art can examine the hydrophobic transmembrane amino acid sequence or sequences of a selected integral membrane protein and identify a suitable amino acid sequence for an antagonist peptide specific for that protein.

The present invention also enables the rational design of specific antagonist peptides or blockers active against the protein product of any gene predicted to encode an integral membrane protein.

Once the amino acid sequence of a new integral membrane protein is determined, for example by cloning and sequencing a gene or cDNA for the protein and

deducing therefrom the amino acid sequence, the amino acid sequence can be subjected to hydropathic analysis, for example using a computer program such as STRIDER, to deduce the transmembrane domains, as is known to those of ordinary skill in the art.

The amino acid sequence of at least one transmembrane domain is then synthesised to provide a selective peptide antagonist of the integral membrane protein.

Alternatively, suitable effective fragments or analogues of a transmembrane amino acid sequence may be selected and screened as described herein.

The present invention enables novel specific pharmaceuticals for treatment of many disorders. Selection of specific antagonist peptides in accordance with the invention enables the development of discriminating drugs with previously unavailable selectivity and hence reduced side-effects.

For example, receptor and transporter antagonists may be used to treat disorders associated with specific receptor overactivity such as schizophrenia which is associated with overactivity of the D2 dopamine receptor, or may be used to indirectly restore homeostasis in disorders which do not directly involve aberrant function of the particular receptor or transporter. Examples of disorders and antagonists in the latter category include: anti-D1 dopamine receptor for drug abuse, anti-histamine receptor for peptic ulcer disease, anti-angiotensin receptor for hypertension and antiβ adrenergic receptor for glaucoma.

Using the D2 dopamine receptor as a model for other membrane spanning receptors, the inventors have shown a dopamine antagonist effect *in vivo*, in a rat model of rotational locomotion, by administering directly into the caudate nucleus of the brain a peptide comprising a

fragment of one of the transmembrane amino acid sequences of the D2 dopamine receptor. Most importantly, the inventors have demonstrated specificity, with no disruption of other closely related receptors by the peptide designed for the D2 dopamine receptor.

In a further animal model, the inventors have shown that a peptide comprising a portion of a transmembrane domain of the β1-adrenergic receptor inhibited the function of that receptor, and a peptide comprising a portion of a transmembrane domain of the α1A-adrenergic receptor inhibited the function of that receptor, as evidenced by the effect of these peptides on cardiac function and blood pressure.

Antagonist peptides in accordance with the invention may be prepared by any suitable peptide synthetic method.

Chemical synthesis may be employed, for example standard solid phase peptide synthetic techniques may be used. In standard solid phase peptide synthesis, peptides of varying length can be prepared using commercially available equipment. This equipment can be obtained from Applied Biosystems (Foster City, CA.). The reaction conditions in peptide synthesis are optimized to prevent isomerization of stereochemical centres, to prevent side reactions and to obtain high yields. The peptides are synthesized using standard automated protocols, using t-butoxycarbonyl-alpha-amino acids, and following the manufacturer's instructions for blocking interfering groups, protecting the amino acid to be reacted, coupling, deprotecting and capping of unreacted residues. The solid support is generally based on a polystyrene resin, the resin acting both as a support for the growing peptide chain, and as a protective group for the carboxy terminus. Cleavage from the resin yields the free carboxylic acid. Peptides are purified by HPLC techniques, for example on a preparative C18 reverse

phase column, using acetonitrile gradients in 0.1% trifluoroacetic acid, followed by vacuum drying.

Antagonist peptides may also be produced by recombinant synthesis. A DNA sequence encoding the desired peptide is prepared, for example by cloning the required fragment from the DNA sequence encoding the complete receptor, obtainable from genomic DNA or from commercially available genomic or cDNA libraries, and subcloning into an expression plasmid DNA. Suitable mammalian expression plasmids include pRC/CMV from Invitrogen Inc. The gene construct is expressed in a suitable cell line, such as a Cos or CHO cell line and the expressed peptide is extracted and purified by conventional methods. Suitable methods for recombinant synthesis of peptides are described in "Molecular Cloning" (Sambrook, Fritsch and Maniatis, Cold Spring Harbor Laboratory Press, 1989).

Analogues of a transmembrane amino acid sequence of an integral membrane protein may be prepared by similar synthetic methods. The term "analogue" extends to any functional and/or chemical equivalent of a transmembrane amino acid sequence and includes peptides having one or more conservative amino acid substitutions, peptides incorporating unnatural amino acids and peptides having modified side chains.

Examples of side chain modifications contemplated by the present invention include modification of amino groups such as by reductive alkylation by reaction with an aldehyde followed by reduction with $NaBH_4$; amidation with methylacetimidate; acetylation with acetic anhydride; carbamylation of amino groups with cyanate; trinitrobenzylation of amino groups with 2, 4, 6, trinitrobenzene sulfonic acid (TNBS); alkylation of amino groups with succinic anhydride and tetrahydrophthalic

anhydride; and pyridoxylation of lysine with pyridoxal-5'-phosphate followed by reduction with $NaBH_4$.

The guanidino group of arginine residues may be modified by the formation of heterocyclic condensation products with reagents such as 2, 3-butanedione, phenylglyoxal and glyoxal.

The carboxyl group may be modified by carbodiimide activation via -acylisourea formation followed by subsequent derivatisation, for example, to a corresponding amide.

Sulfhydryl groups may be modified by methods such as carboxymethylation with iodoacetic acid or iodoacetamide; performic acid oxidation to cysteic acid; formation of mixed disulphides with other thiol compounds; reaction with maleimide, maleic anhydride or other substituted maleimide; formation of mercurial derivatives using 4-chloromercuribenzoate, 4-chloromercuriphenylsulfonic acid, phenylmercury chloride, 2-chloromercuric-4-nitrophenol and other mercurials; carbamylation with cyanate at alkaline pH.

Tryptophan residues may be modified by, for example, oxidation with N-bromosuccinimide or alkylation of the indole ring with 2-hydroxy-5-nitrobenzyl bromide or sulphonyl halides. Tyrosine residues may be altered by nitration with tetranitromethane to form a 3-nitrotyrosine derivative.

Modification of the imidazole ring of a histidine residue may be accomplished by alkylation with iodacetic acid derivatives of N-carbethoxylation with diethylpyrocarbonate.

Examples of incorporating unnatural amino acids and derivatives during peptide synthesis include, but are not limited to, use of norleucine, 4-amino butyric acid, 4-amino-3-hydroxy-5-phenylpentanoic acid, 6-aminohexanoic acid-, t-butylglycine, norvaline, phenylglycine,

ornithine, sarcosine, 4-amino-3-hydroxy-6-methylheptanoic acid, 2-thienyl alanine and/or D-isomers or amino acids.

Examples of conservative amino acid substitutions are substitutions within the following five groups of amino acids:

Group 1: F Y W
Group 2: V L I
Group 3: H K R
Group 4: M S T P A G
Group 5: D E

Fragments or analogues of the antagonist peptides of the invention may be conveniently screened for their effectiveness as receptor antagonists, for example by examining their ability to inhibit ligand-binding by the relevant receptor which has been pre-incubated with the peptide. Ligand-binding inhibition can be determined, for example, by a soluble receptor radioligand binding assay, based on the soluble receptor binding assay described in Example 1 or by a membrane radioligand binding assay as described in Example 3.

The peptide antagonists of the invention may be administered therapeutically by injection or by oral, nasal, buccal, rectal, vaginal, transdermal or ocular routes in a variety of formulations, as is known to those in the art.

For oral administration, various techniques can be used to improve stability, based for example on chemical modification, formulation and use of protease inhibitors. Stability can be improved if synthetic amino acids are used, such as peptoids or betidamino acids, or if metabolically stable analogues are prepared.

Formulation may be, for example, in water/oil emulsion or in liposomes for improved stability. Oral administration of peptides may be accompanied by protease inhibitors such as aprotinin, soybean trypsin inhibitor

or FK-448, to provide protection for the peptide. Suitable methods for preparation of oral formulations of peptide drugs have been described, for example, by Saffran et al., 1979) (use of trasylol protease inhibitor); Lundin et al. (1986) and Vilhardt et al., (1986).

Due to the high surface area and extensive vascular network, the nasal cavity provides a good site for absorption of both lipophilic and hydrophilic drugs, especially when coadministered with absorption enhancers. The nasal absorption of peptide-based drugs can be improved by using aminoboronic acid derivatives, amastatin, and other enzyme inhibitors as absorption enhancers and by using surfactants such as sodium glycolate, as described in Amidon et al., (1994).

The transdermal route provides good control of delivery and maintenance of the therapeutic level of drug over a prolonged period of time. A means of increasing skin permeability is desirable, to provide for systemic access of peptides. For example, iontophoresis can be used as an active driving force for charged peptides or chemical enhancers such as the nonionic surfactant n-decylmethyl sulfoxide (NDMS) can be used.

Transdermal delivery of peptides is described in Amidon et al. (1994) and Choi et al. (1990).

Peptides may also be conjugated with water soluble polymers such as polyethylene glycol, dextran or albumin or incorporated into drug delivery systems such as polymeric matrices to increase plasma half-life.

More generally, formulations suitable for particular modes of administration of peptides are described, for example, in Remington's Pharmaceutical Sciences, latest edition, Mack Publishing Company (Easton, PA.)

The peptide antagonists of the invention also provide a tool for the elucidation of the function of

many important orphan receptors whose structures and locations are known but for which the endogenous ligand is unknown. Disruption of the function of an orphan receptor by a transmembrane peptide antagonist and observation of the resulting loss or disruption of function will assist in elucidating the role of the orphan receptor.

The present invention also provides new methods of tissue imaging. An antagonist peptide derived from the transmembrane amino acid sequence of a membrane-spanning protein may be labelled with a suitable signalling moiety, such as an imaging radionuclide, and administered *in vivo*.

The labelled peptide binds stably to the receptor permitting visualisation and quantification of the receptor.

Suitable radionuclides include technetium^{99}, thallium, ^{11}C or ^{18}F.

Peptides may be labelled by conventional methods known to those skilled in the art.

The specificity of the antagonist peptides of the invention for the receptor will provide improved accuracy and precision in the determination of receptor localisation and receptor density on cells and in tissues. Detection of the signalling moiety and therefore of the bound receptor can be carried out by conventional methods suitable for each particular labelling moiety.

In a further embodiment, the invention provides new methods for gene therapy utilising a genetically engineered, recombinant nucleotide sequence encoding a peptide antagonist, incorporated in a suitable transfection vector for introduction of the coding sequence into a selected cell or tissue, either *in vitro*

or *in vivo*, in order to provide for *in vivo* production of a selected integral membrane protein antagonist.

In a further embodiment, the invention provides transgenic animal models expressing transmembrane peptide antagonists which modulate endogenous integral membrane protein function. These animal models will provide a tool for testing the design, efficacy and toxicology of integral membrane protein antagonist peptides and will also provide models that mimic clinical diseases.

Transgenic animal models in accordance with the invention can be created by introducing a DNA sequence encoding a selected peptide antagonist either into embryonic stem cells (ES) of a suitable animal, for example a mouse, by transfection or microinjection, or into a germ line or stem cell by a standard technique of oocyte microinjection.

The ES cells are inserted into a young embryo and this embryo or an injected oocyte are implanted into a pseudo-pregnant foster mother to grow to term.

The techniques for generating transgenic animals are now widely known and are described in detail, for example, in Hogan et al., (1986), and M. Capecchi (1989).

1. G-Protein Coupled Receptor Antagonists

The G-protein coupled receptors have a common pattern of seven hydrophobic membrane-spanning domains. These receptors are involved in a wide variety of pathways. Table 1 lists the various receptors which belong to this superfamily.

(a) Dopamine Receptor Antagonists

In accordance with one embodiment of the present invention, a method is provided for regulating or inhibiting the activity of a selected dopamine receptor.

Five distinct dopamine receptors have been identified and are designated D1 to D5 (Seeman, P.

(1995). All belong to the family of G protein-coupled receptors (GPCRs) that have seven highly conserved membrane spanning regions which are linked by intracellular and extracellular loops (O'Dowd, 1993). Comparison of the primary structure of GPCRs shows that the greatest similarity exists in the transmembrane domains, whereas greatest differences are found within the N- and C-terminal regions and the cytoplasmic third loop connecting transmembrane domains V and VI. For instance, D1 and D5 receptors have a shorter third intracellular loop and a longer carboxyl tail compared to D2, D3, and D4 receptors.

One of ordinary skill in the art is enabled by this invention to identify specific antagonist compounds which regulate or inhibit each of the dopamine receptors.

(i) Dopamine D2 receptor

The following description of the dopamine D2 receptor and antagonists of the receptor illustrates the selection of a specific antagonist peptide to an integral membrane protein, in accordance with the invention.

The dopamine D2 receptor is activated by the neurotransmitter, dopamine, leading to the inhibition of intracellular adenylate cyclase.

The D2 receptor gene encodes a long and a short form of the receptor, differing by a 29 amino acid segment in the third intracellular loop. The long and short forms have identical transmembrane domains.

Figure 1 shows a two-dimensional representation of the D2 dopamine receptor spanning the cell membrane. The seven transmembrane domains and two cytoplasmic domains are identified, the transmembrane amino acid sequences being shown in bold.

Table 2 shows the information available in the SwissProt database for the dopamine D2 receptor, Accession No. P14416. (SEQ ID NO:8)

The transmembrane domains are identified by amino acid number, as follows:

TM 1	amino acids 38 to 60
TM 2	amino acids 72 to 97
TM 3	amino acids 109 to 130
TM 4	amino acids 152 to 174
TM 5	amino acids 187 to 210
TM 6	amino acids 374 to 397
TM 7	amino acids 406 to 429

The amino acid sequences of these transmembrane domains can be determined from the complete amino acid sequence provided. Any one of these transmembrane amino acid sequences may be selected for use as a specific antagonist of the D2 dopamine receptor.

For the D2 dopamine receptor, therefore, an antagonist peptide may be selected from the following transmembrane amino acid sequences:

A

TM I	ATLLTLLIAVIVFGNVLVCMAVS (SEQ ID NO:1)
TM II	LIVSLAVADLLVATLVMPWVVYLEVV (SEQ ID NO:2)
TM III	IVFTLDVMMCTASILNLCAISI (SEQ ID NO:3)
TM IV	VTVMISIVWVLSFTISCPLLFGL (SEQ ID NO:4)
TM V	PAFVVYSSIVSFYVPFIVTLLVYI (SEQ ID NO:5)
TM VI	MLAIVLGVFIICWLPFFITHILN (SEQ ID NO:6)
TM VII	VLYSAFTWLGYVNSAVNPIIYTTF (SEQ ID NO:7)

or may be an effective fragment or analogue of any of these sequences.

D2 receptors are found in brain, where the highest densities have been found in the striatum (Caudate-putamen, nucleus accumbens), olfactory tubercle, and substantia nigra and pituitary, whereas lower densities are present in the cortex, hippocampus and limbic brain regions (Bouthenet et al., 1987, 1991, Mansour et al., 1990). At the cellular level, D2-like receptors have been identified on synaptic nerve terminals, and there is evidence that D2 receptors are colocalized with D1

receptors in certain neuronal populations (Surmeier et al., 1992). These mapping studies also indicate a pre-synaptic localization of D2-like receptors where they may function as autoreceptors regulating the synthesis and/or release of dopamine (Starke et al., 1989, Sokoloff et al., 1990).

D2 receptors have also been localized in mammals to the femoral artery, renal and mesenteric arteries, adrenal chromaffin cells (indicating involvement in cardiovascular homeostasis) and retina.

Abnormal functioning of D2 receptors has been implicated in a number of neuropsychiatric diseases and in substance abuse; existing antipsychotic drugs have been shown to selectively block the D2 receptor.

Dopamine D2 receptor blockers are therefore main candidates for neuroleptic drugs used in the treatment of neuropsychiatric disease such as schizophrenia, Huntington's disease and Tourette's syndrome (Seeman and Niznik, 1990). They may also provide alternative treatments for substance abuse (Ng and George, 1994c; George et al., 1995).

It is highly desirable to be able to inhibit or regulate the activity of the D2 receptor without affecting the other four dopamine receptors. This degree of selectivity has not so far been possible with the previously available dopamine receptor antagonists.

The inventors have shown that by selecting a transmembrane domain of the D2 receptor and preparing a peptide of amino acid sequence corresponding to all or a portion of the selected transmembrane domain, one can obtain a highly specific antagonist of D2 receptor activity.

A peptide corresponding to a portion of a transmembrane domain of the D2 dopamine receptor did not cause dissociation of the D1 dopamine receptor or the

serotonin 5HT1B receptor but did cause dissociation of the D2 dopamine receptor dimer, whereas a peptide corresponding to the cytoplasmic domain of the D2 receptor did not cause dissociation of the D2 receptor. A peptide corresponding to a transmembrane domain of the β2-adrenergic receptor did not cause dissociation of the D2 dopamine receptor dimer.

The selectivity of the antagonist peptides of the invention surpasses that of presently available agents for blocking D2 receptor activity.

(ii) Dopamine D1 and D3 to D5 receptors

The dopamine D1 and D3 to D5 receptors are also receptors for the neurotransmitter dopamine and are found in brain as well as in other tissues.

The amino acid sequences of these receptors, and identification of their transmembrane domains, can be obtained from SwissProt Database under the Accession Numbers listed in Table 3.

Using this information, one skilled in the art can readily ascertain the transmembrane amino acid sequences for each of these dopamine receptors and can thereby synthesise antagonist peptides suitable and specific for each receptor. For example, for the D1 dopamine receptor, an antagonist peptide may be selected from the following transmembrane amino acid sequences:

TM 1	ILTACFLSLLILSTLLGNTLVCAAV	(SEQ ID NO:9)
TM 2	FFVISLAVSDLLVAVLVMPWKAVAEIA	(SEQ ID NO:10)
TM 3	NIWVAFDIMCSTASILNLCVISVD	(SEQ ID NO:11)
TM 4	AAFILISVAWTLSVLISFIPVQLSW	(SEQ ID NO:12)
TM 5	TYAISSSVISFYIPVAIMIVTYTRI	(SEQ ID NO:13)
TM 6	TLSVIMGVFVCCWLPFFILNCILPFC	(SEQ ID NO:14)
TM 7	FDVFVWFGWANSSLNPIIYAFNAD	(SEQ ID NO:15)

Fragments and analogues of these peptides may be selected and screened as described herein.

The D1 dopamine receptor has been associated with drug abuse and the D3 and D4 receptors have been associated with schizophrenia. Antagonists of these receptors in accordance with the invention provide specific therapeutic agents for use in these conditions.

(b) Adrenergic Receptors

The adrenergic receptors (AR) mediate the effects of the catecholamines epinephrine and norepinephrine on a wide variety of physiologic processes, such as regulation of blood pressure and heart rate.

Molecular cloning studies have now shown that the physiological actions of epinephrine and norepinephrine are mediated by the products of at least nine types of AR. These receptors can be subdivided into three groups, each capable of coupling to different G proteins. β_1-, β_2-, and β_3AR are involved in the activation of adenylyl cyclase. Conversely, activation of the platelet and kidney α_2AR (α_2AR-C10 and α_2AR-C4, respectively) inhibits adenylyl cyclase activity *via* the intermediacy of the G protein G_i. The α_1 AR receptors (α_{1A} and α_{1B}) have the ability to stimulate phospholipase C. Stimulation of this effector enzyme leads to membrane phospholipid hydrolysis and the subsequent mobilization of calcium from intracellular stores.

The various AR, and the respective G proteins to which they couple, provide the means by which the two adrenergic agonists epinephrine and norepinephrine can elicit many different intracellular responses.

The amino acid sequences of these receptors, and identification of their transmembrane domains, can be obtained, for example, from SwissProt database under the Accession Numbers listed in Table 3.

For example, for the β1-adrenergic receptor, an antagonist peptide may be selected from the amino acid sequences:

TM I	GMGLLMALIVLLIVAGNVLVIVAI	(SEQ ID NO:16)
TM II	IMSLASADLVMGLLVVPFGATIVV	(SEQ ID NO:17)
TM III	ELWTSVDVLCVTASIETLCVIALD	(SEQ ID NO:18)
TM IV	RGLVCTVWAISALVSFLPILMHWW	(SEQ ID NO:19)
TM V	RAYAIASSVVSFYVPLCIMAFVYL	(SEQ ID NO:20)
TM VI	LGIIMGVFTLCWLPFFLANVVKAF	(SEQ ID NO:21)
TM VII	RLFVFFNWLGYANSAFNPIIYCRS	(SEQ ID NO:22)

For the α1A-adrenergic receptor, an antagonist peptide may be selected from the amino acid sequences:

TM I	GVGVGFLAAFILMAVAGNLLVILSV	(SEQ ID NO:23)
TM II	FIVNLAVADLLLSATVLPFSATMEVL	(SEQ ID NO:24)
TM III	DVWAAVDVLCCTASILSLCTISV	(SEQ ID NO:25)
TM IV	AAILALLWVVALVVSVGPLLGWKEP	(SEQ ID NO:26)
TM V	AGYAVFSSVCSFYLPMAVIVVMYC	(SEQ ID NO:27)
TM VI	LAIVVGVFVLCWFPFFFVLPLGSL	(SEQ ID NO:28)
TM VII	EGVFKVIFWLGYFNSCVNPLIYPCS	(SEQ ID NO:29)

Fragments and analogues of these peptides may be selected and screened as described above.

The inventors have shown that the peptide FFNWLGYANSAFNP$_A$, (SEQ ID NO:30) a fragment of the TM VII domain of the human β1-adrenergic receptor, inhibited the function of that receptor. The inventors have also shown that the peptide VFKVIFWLGYFNSCVN$_A$, (SEQ ID NO:31) a fragment of the TM VII domain of the human α1A-adrenergic receptor, inhibited the function of that receptor.

Adrenergic receptor antagonists are accepted therapeutic agents for treatment of hypertension. The adrenergic receptor antagonist peptides of the present invention provide new agents with previously unavailable specificity.

(c) Adenosine Receptors

Adenosine is a neuromodulator which is released in response to increased activity or stress.

Adenosine receptors are found in both central and peripheral neural locations. Four subtypes of adenosine receptors, designated A1, A2a, A2b and A3, have been identified. Their sequences and transmembrane domains are available, for example, under the Accession Numbers listed in Table 3.

Using this sequence information, specific antagonist peptides can be identified and prepared, as described elsewhere herein.

In general, adenosine exerts a depressant action in the brain, heart and kidneys by activating adenosine receptors. The depressant action in the brain is believed to confer neuroprotection. Moreover, centrally acting adenosine has been shown to be involved in pain, cognition, movement and sleep. Peripherally, adenosine is believed to have arrhythmic, hypotensive and antilipolytic properties.

The antiasthmatic effects of theophylline and the antidepressant and cognition-enhancing effects of caffeine are attributed to their action as adenosine receptor antagonists.

Adenosine receptor antagonists have a role as therapeutics in the treatment of cardiovascular, renal and central nervous system disorders and are likely to be useful as anti-asthmatics, anti-depressants, anti-arrhythmics, anti-Parkinsonian therapeutics, cognitive enhancers and as renal protective agents.

2. Tyrosine Kinase Receptor Antagonists

The tyrosine kinase receptors have an amino terminus involved in ligand binding, a single membrane-spanning domain and a homologous carboxyl tail catalytic domain

with intrinsic tyrosine kinase activity (Kraus et al., 1989).

Examples of such tyrosine kinase receptors include receptor families for a number of growth factors, including epidermal growth factor (EGF), colony-stimulating factor 1/platelet derived growth factors and insulin/insulin-like growth factor.

The amino acid sequence and transmembrane domain of EGF is available, for example, under the Accession Numbers listed in Table 3.

From this information, one skilled in the art can readily ascertain the transmembrane amino acid sequence of EGF: PSIATGIVGGLLFIVVVALGIGL (SEQ ID NO:32)

This amino acid sequence may be used as antagonist peptide or fragments or analogues may be selected and screened as described above.

EGF receptors are localized in a wide range of epithelial and fibroblastic cells.

Binding of EGF to the extracellular portion of the receptors results in an association of two EGF receptor molecules (dimerization) that leads to conformational changes resulting in the phosphorylation of the cytoplasmic domain of the receptor (Boni-Schnetzler et al., 1987).

Growth factor receptors have been implicated in the development of cancer. In particular, EGF receptors have been shown to act as oncogenes by mechanisms of overexpression or mutations that constitutively activate the intrinsic tyrosine kinase activity of these proteins (Schlessinger, J., 1986; Yarden et al., 1987).

The ability to inhibit or regulate activity of these receptors by the antagonist peptides of the invention provides a new, specific tool to prevent the development of, or control, neoplastic growth and cancer.

3. Ion Channel and Channel Protein Antagonists

A number of integral membrane proteins form ion channels or ion channel receptors or are channel proteins.

One example is the γ-aminobutyric acid (GABA)-A receptor-chloride ion channel complex, which belongs to the ligand-gated receptor superfamily, which also includes the 5HT3 serotonin receptor, the nicotinic acetylcholine receptor and the metabotropic glutamate receptor.

The GABA-A receptor-chloride ion channel is believed to be a complex of five membrane-spanning protein subunits forming a heteroligomer. The subunits belong to α, β, γ, δ or ρ class. Each subunit has an N-terminus, 4 putative hydrophobic membrane spanning domains, and a C-terminus, linked by extracellular and intracellular loops (Schofield et al., (1987); Bernard, E.A., 1995).

The amino acid sequences of the GABA-A subunits are provided, and the transmembrane hydrophobic sequences are identified, for example, under the Accession Numbers listed in Table 3.

Using this information, one of ordinary skill in the art can readily identify, for example, the transmembrane amino acid sequences for the human α1-subunit which are as follows:

TM1 YFVIQTYLPCIMTVILSQVSFW (SEQ ID NO: 33)
TM2 VPARTVFGVTTVLTMTTLSISA (SEQ ID NO:34)
TM3 MDWFIAVCYAFVFSALIEFATV (SEQ ID NO: 35)
TM4 LSRIAFPLLFGIFNLVYWATYL (SEQ ID NO:36)

An antagonist peptide for GABA-A receptor may be selected from these sequences or from the transmembrane sequences of any of the other subunits of the receptor.

Effective fragments or analogues of any of these peptides may be selected and screened as described herein.

GABA is the principal inhibitory neurotransmitter in the vertebrate brain which mediates its actions (neuronal inhibition) by binding to the integral membrane protein, the GABA-A receptor. GABA-A receptors form a fast-acting ligand-gated chloride ion-selective channel, that upon activation by agonist, results in the hyperpolarization of the neuron.

GABA-A receptor channels mediate the major inhibitory synaptic events in the brain and are involved in the regulation of anxiety, vigilance, memory, epileptogenic activity and muscle tension. GABA-A receptor subtypes have been identified in hippocampus (Pyramidal and interneurons), olfactory bulbs (Mitral and Granule cells), thalamus (relay neurons and Reticular nucleus), and in the cerebellum (Purkinje and Granule cells).

A number of drugs which have their effect on the brain act by binding to the GABA agonist site or receptor channel; these include benzodiazepines which are anxiolytic, barbiturates which are anti-convulsant, β-carbolines which are anxiogenic and picrotoxin which has convulsant effects.

4. Transporter Antagonists

The family of transporter proteins are glycoproteins with twelve putative membrane-spanning domains which mediate sodium- and chloride-dependent re-uptake of neurotransmitter.

The neurotransmitter transporter proteins provide a re-uptake mechanism for neurotransmitters, thereby inactivating released transmitter. This is the most important mechanism for terminating synaptic transmission of endogenous ligands such as catecholamines.

The dopamine transporter is described by way of example of this family of integral membrane proteins.

The amino acid sequence and transmembrane domains of the dopamine transporter are available, for example, under the Accession Number listed in Table 3.

Using this information, one skilled in the art can readily ascertain that an antagonist peptide for the dopamine transporter may be selected from the following transmembrane amino acid sequences:

T1 FLLSVIGFAVDLANVWRFPYL (SEQ ID NO:37)
T2 GAFLVPYLLMVIAGMPLFYM (SEQ ID NO:38)
T3 GVGFTVILISLYVGFFYNVII (SEQ ID NO:39)
T4 WQLTACLVLVIVLLYFSLW (SEQ ID NO:40)
T5 VVWITZTMPYVVLTALLL (SEQ ID NO:41)
T6 VCFSLGVGFGVLIAFSSY (SEQ ID NO:42)
T7 IVTTSINSLTSFSSGFVVFSFL (SEQ ID NO:43)
T8 LPLSSAWAVVFFIMLLTGLI (SEQ ID NO:44)
T9 LFTLFIVLATFLLSLFCVT (SEQ ID NO:45)
T10 GTSILFGVLIEAIGVAWFYGV (SEQ ID NO:46)
T11 LCWKLVSPCFLLFVVVVSIV (SEQ ID NO:47)
T12 LGWVIATSSMAMVPIYAAY (SEQ ID NO:48)

Effective fragments and analogues of these peptides may be selected and screened as described herein.

The distribution of transporters is consistent with the distribution of neurotransmitters, suggesting that transporters might be expressed specifically for the neurotransmitter system. Transporter localization is chiefly in the presynaptic neuronal membrane.

There are also high affinity transporter proteins for norepinephrine, glutamate, aspartate, GABA, glycine, taurine, proline, adenosine and serotonin 5-HTT.

Abnormalities of the transporter proteins have been linked to several neuropsychiatric disorders (Uhl et al., (1994), J. Exp. Biol. v. 196, pp. 229-236).

The dopamine transporter and other monoamine transporters are the target of major classes of antidepressant and psychostimulant drugs. The dopamine

transporter is also targeted by drugs of abuse such as cocaine and amphetamine.

The antagonist peptides of the invention provide new, specific therapeutic agents useful in these dopamine transporter-related disorders as antidepressants and for the relief of drug craving and dependence.

8. Antigen Receptor Antagonists

A number of eukaryotic cell types have membrane-associated antigen receptors which are integral membrane proteins.

T cells, for example, have the T cell antigen receptor which provides the molecular basis for major histocompatability complex (MHC) antigen recognition. The receptor consists of two linked glyco-peptides, one of which, the α-glycopeptide, consists of a transmembrane domain and a cytoplasmic domain.

The amino acid sequence of the human T cell antigen receptor α chain is provided, for example, in Yoshikai et al. (1985), the contents of which are incorporated herein by reference.

Using this information, one of ordinary skill in the art can readily identify the transmembrane amino acid sequence which can be used as an antagonist of the T cell antigen receptor:

DTNLNFQNLSVIGFRILLLKVAGFNLLMTLRLWSS (SEQ ID NO:49)

Effective fragments or analogues of this peptide may be selected and screened as described herein.

The human immunodeficiency virus (HIV) is known to attach itself to the T cell antigen receptor and thereby gains access to the cell. An antagonist peptide based on the amino acid sequence of the transmembrane domain of the T cell receptor, in accordance with the invention, provides a therapeutic agent which can disrupt the

function of the T cell receptor and prevent access of the HIV virus to the T cell.

EXAMPLES

The examples are described for the purposes of illustration and are not intended to limit the scope of the invention.

Methods of molecular genetics, protein and peptide biochemistry and immunology referred to but not explicitly described in this disclosure and examples are reported in the scientific literature and are well known to those skilled in the art.

Materials: The mouse monoclonal antibody anti-c-myc (9E10), and the D2 antisera were obtained from Santa Cruz Biotechnology. The genes expressing receptors were obtained by standard molecular biology techniques (Maniatis), as described below. The human D2L receptor cDNA was from Dr. O. Civelli (Portland, OR, USA). All other chemicals and materials were of highest analytical grade and purchased from commercial sources.

Peptides: The following fragments or analogues of the indicated domains of various receptors were synthesised by Chiron Mimotopes Peptide Systems, (Raleigh, N.C.) using solid state peptide synthesis as described in Geysen et al., (1984) or by QCB Inc. (Maryland, U.S.A.):

Human β2 adrenergic receptor: free amine (H)-peptide-free acid (COOH)

β2-AR-TM VI H-GIIMGTFTLCWLPFFIVNIVH-COOH (SEQ ID NO:50)

Human serotonin 5-HT1B receptor: free amine (H)-peptide free amide (NH2)

5-HT1B-TM VII H-FHLAIFDFFTWLGYLNSLIN-NH2 (SEQ ID NO:51)

Rat α1A adrenergic receptor: free amine (H)-peptide-free acid (COOH)

α1A-AR-TM VII H-VFKVIFWLGYFNSCVN-COOH (SEQ ID NO:31)

Rat β1 adrenergic receptor: free amine (H)-peptide-free acid (COOH)

β1-AR-TM VII H-FFNWLGYANSAFNP-COOH (SEQ ID NO:30)

Rat angiotensin 1 receptor: free amine (H)-peptide-free acid (COOH)

AT1-TM VII H-VDTAMPITICIAYFNN-COOH (SEQ ID NO:52)

Rat vasopressin 2 receptor: free amine (H)-peptide-free acid (COOH)

V2-TM VII H-LMLLASLNSCTNPWIY-COOH (SEQ ID NO:53)

Human EGF receptor: free amine (H)-peptide-free acid (COOH)

EGF-TM I H-LTVIAGLVVIFMMLGG-COOH (SEQ ID NO:54)

Human dopamine D1 receptor: free amine (H)-peptide-free acid (COOH)

Human dopamine D2 receptor: free amine (H)-peptide-free amine (NH_2)

D2-TM-VII H-LYSAFTWLGYVNSAVNPIIY-NH2 (SEQ ID NO:55)

D2-TM VI H-LAIVLGVFIICWLPFFITHI-NH2 (SEQ ID NO:56)

D2-TM V H-PAFVVYSSIVSFYVPFIVTL-NH2 (SEQ ID NO:57)

D2-TM IV H-RVTVMISIVWVLSFTISCPL-NH2 (SEQ ID NO:58)

D2-TM III H-TLDVMMCTASILNLCAISID-NH2 (SEQ ID NO:59)

D2-TM II H-VSLAVADLLVATLVMPWVVY-NH2 (SEQ ID NO:60)

D2-TM I H-YATLLTLLIAVIVFGNVLVC-NH2 (SEQ ID NO:61)

D2-C IIIA H-CTHPEDMKLCTVIMKSNGSF-NH2 (SEQ ID NO:62)

D2-C IIIB H-LSSTSPPERTRYSPIPPSHH-NH2 (SEQ ID NO:63)

Example 1 - Dopamine D2 receptor

Recombinant D2 receptor: Recombinant baculovirus encoding the human D2L receptor was constructed using standard recombinant techniques.

Briefly, a cDNA clone encoding the long form of the human D2 receptor (D2L) was extracted from the pZem 3 vector with *Dra*III and *Kpn*I. The resulting fragment was blunt ended and isolated by electrophoresis on soft agarose. The transfer vector pJVETLZ New was digested with *Neh*I and blunt ended. The cDNA fragment coding for the D2L receptor was inserted into this vector by blunt-end ligation and the orientation verified by sequencing.

Transfer of recombinant baculovirus encoding the D2L receptor into the AcNPV genome was achieved by co-transfection of plasmid and wild-type viral DNA in Sf9 cells using the calcium phosphate precipitation procedure. Purification of recombinant virus was carried out as described by Vialard et al., 1990, and stocks of the purified recombinant viruses were amplified in Sf9 cells.

Sf9 Cell Culture: Sf9 cells were grown in monolayer or suspension culture essentially as described by Summers and Smith (1987) in supplemented Grace's insect media at 27 C. Pluronic F-68, a cell protective agent, was added to improve cell viability in suspension culture since Sf9 cells are very sensitive to mechanical shear. Cells at a density of 1-2 x 10^6/ml were infected with the recombinant virus at a multiplicity of infection of 2-5 and harvested at 24 or 48 h post-infection. Typically, viral infections were performed with a final cell density of 2 x 10^6 cells/ml. Cell viability at these times were ~90% (trypan blue-negative) as determined by trypan blue staining. To minimize variations in recombinant baculovirus infection efficiencies among batches of cultures, the same recombinant virus stock was used in all experiments for a study.

Preparation of Sf9 Cell Membrane Fractions: The preparation of membranes was done at 4°C. Cells were centrifuged at 100xg for 7 min. and pelleted. Cells were then washed twice with PBS and centrifuged at 100xg for 7 min (X2) and resuspended in 10 ml of buffer A: 5 mM Tris-HCl, 2 mM EDTA buffer containing the protease inhibitors: 10μg/ml benzamidine, 5 μg/ml leupeptin, and 5 μg/ml soybean trypsin inhibitor (pH 7.4 at 4°C). The cell suspension was then sonicated, two bursts at maximum setting for 15 seconds and homogenates were centrifuged at 100xg for 7 min. to pellet unbroken cells and nuclei, and supernatant was collected. The resulting pellet was homogenized a second time in 10 ml of buffer A, centrifuged as described above and supernatant fractions saved. The pooled S1 supernatant was centrifuged at high speed (27,000 xg for 20 min), washed once with buffer A, centrifuged again at high speed and resuspended in buffer A, and stored at -80 C or resuspended in buffer B: 75 mM Tris-HCl, 12.5 mM $MgCl_2$, 2 mM EDTA, pH 7.4 and assayed immediately for adenylyl cyclase activity. Pelleted membranes were resuspended in buffer A and stored at -70° C or resuspended in the appropriate buffers for immediate use in various assays.

Solubilization And Immunoprecipitation Of Receptors: Membranes were prepared by sonication in buffer A as described above. The pellet was resuspended and stirred at 4°C for 2 h in 2 ml of freshly prepared solubilization buffer consisting of 100 mM NaCl, 10 mM Tris-HCl pH 7.4, 2% digitonin, and 5 mM EDTA with protease inhibitors. The homogenate was centrifuged at 27000xg for 20 min and the solubilized fraction was washed and concentrated in Centriprep 30 four times with 10 ml cold buffer C: 100 mM NaCl, 10 mM Tris-HCl pH 7.4 with protease inhibitors. The washed fraction was precleared with 1/20 normal

rabbit serum and protein A-Sepharose beads for 2 h on ice. Solubilized receptors were immunoprecipitated with the mouse monoclonal 9E10 antibody (D1 receptor) or rabbit polyclonal (D2 receptor) at a 1/37 dilution in buffer C for 2 h on ice, and agitated gently overnight at 4°C with 1/40 dilution of agarose fixed goat anti-primary IgG. The immunoprecipitate was washed 6 times with 5 volumes cold buffer C for 20 min, solubilized in SDS sample buffer, sonicated and electrophoresed on SDS-PAGE as described above.

Protein Determination: Protein content was determined by the method of Bradford (BioRad). A standard protein concentration curve was made with bovine serum albumin (BSA). Protein concentration in the test sample was determined from the standard curve which was a plot of absorbance at 595 nm measured using a Hitachi model U-2000 spectrophotometer against concentration.

Soluble Receptor Binding: The amount of solubilized and immunoprecipitated receptor was determined by radioligand binding. D2 receptor density was estimated by incubating in the presence of saturating concentrations (~2000 pM) of the benzamide antagonist [^{3}H]YM-01951-2 for 2 h at 22°C in a total volume of 1 ml binding buffer: 100 mM NaCl, 10 mM Tris-HCl, 0.05% digitonin, 2mM EDTA with protease inhibitors, pH 7.4. Nonspecific binding was defined by binding not displaced by 1 μM (+)butaclamol. Following the incubation period, the binding preparation was loaded onto a Sephadex G-50 column (Pharmacia) and ligand bound receptor was separated from free ligand by elution. The eluate was counted in a scintillation counter for determination of the amount (pM) of receptor.

SDS-PAGE Electrophoresis: Sodium dodecyl sulphate 10-12% polyacrylamide gel electrophoresis (SDS-PAGE) was performed as described by Laemmli, 1970. Immunoprecipitated membranes were solubilized in SDS sample buffer consisting of 50 mM Tris-HCl pH 6.5, 10% SDS, 10% glycerol, and 0.003% bromophenol blue with or without 10% 2-mercaptoethanol. Molecular mass (Da) of receptors was determined graphically by plotting the log molecular weight of known protein standards versus the RF (relative migration) of these proteins. The apparent molecular mass of proteins was estimated by determining the RF (from the centre of the band) and interpolating this value onto the standard curve.

Immunoblot Analysis: Membranes from cells infected with recombinant virus or with wild-type baculovirus were prepared and subjected to SDS-PAGE electrophoresis as described above and blotted on to nitrocellulose. The blots were washed in TBS for 10 min, blocked with 3% skim milk powder in TBS buffer for 30 min, washed for 10 min and incubated for 1 h at 22°C with the mouse monoclonal antibody (9E10) directed against the c-myc epitope of the c-myc-D1 receptor, or with the polyclonal antibody against a 120 amino acid sequence (nt 661-1020) in the third intracellular loop of the human D2 receptor. Primary antibodies were diluted 1/1000 in TBS containing 1% skim milk powder. Blots were then treated with 0.05% Tween 20 in TBS for 30 min and binding of the primary antibody was detected after incubation for 1 h at 22 C with goat anti-mouse or goat anti-rabbit IgG alkaline phosphatase conjugate diluted 1/1000 in TBS containing 1% skim milk powder. Blots were then rinsed in 150 mM NaCl, 50 mM Tris-HCl pH 7.5 before developing with BCIP/NBT (5-bromo-4-chloro-3-indolyl phosphate/nitroblue terazolium) substrate.

Preparation of human caudate tissue:

Human caudate tissue was obtained from the Canadian Brain Tissue Bank and cell membranes were isolated and receptors solubilised and immunoprecipitated as described above.

In Vitro Receptor knock-out studies: 3 pmol of isolated and solubilised receptors, as determined by soluble binding assay, were prepared in buffer A (100 mM NaCl, 10 mM Tris-HCl pH 7.4, mM EDTA and 0.5% digitonin with 5 μg/ml leupeptin, 10 μg/ml benzamidine and 5 μg/μl soybean trypsin inhibitor), and incubated with 1.6 g/l (final concentration) of peptide (unless otherwise indicated). All peptides were freshly prepared in buffer A with 10% DMSO. SDS buffer (50 mM Tris-HCl, pH 6.5/12% SDS/10% 2-mercaptoethanol/10% (vol/vol) glycerol/0.003% bromophenol blue) was added to the samples to make a total assay volume of 30 μl, and incubated at 37°C for 30 min prior to SDS-PAGE and immunoblot analysis.

Receptor temperature stability studies were performed with 1.5 pmol of immunoprecipitated D2 receptors. Receptors were prepared in buffer A and incubated at 23, 37, 65, and 90°C for 30 min with SDS buffer at a final volume of 30 μl, and subjected to SDS-PAGE and immunoblot analysis.

Receptor pH-stability experiments were performed with 1.5 pmol of immunoprecipitated D2 receptors prepared in buffer A. Receptors were treated with H_2O, or 0.1 N tartaric acid (final concentration), or 0.1 N HCL (final concentration), or 0.1% glacial acetic acid (final concentration). Samples were then incubated at 37°C for 30 min with SDS buffer at a final volume of 30 μl, and subjected to SDS-PAGE and immunoblot analysis.

Consistent with the model that D2 receptors exist as dimers, D2 dimers dissociate as a function of increasing

temperature (Figure 2F) or in the presence of acid (approximate pH 3.0) (Figure 2G).

Peptide-induced specific disruption of receptors. D2 receptors immunoprecipitated from membranes prepared from D2/Sf9 cells exist in the presence of sodium dodecyl sulphate (SDS) and reducing agent (2-mercaptoethanol) as ~44 kDa species at the predicted size of the D2 receptor, and as ~90 kDa species at approximately twice the molecular mass interpreted to be D2 dimers (Fig. 2A, lane 1). The dissociation of the D2 dimer to monomer was accomplished in a dose-dependent manner upon addition of the hydrophobic peptide LAIVLGVFIICWLPFFITHI$_R$ (SEQ ID NO:56) aa 375-394 of the D2 receptor, within the TM VI domain(Fig. 2A) or the peptide LYSAFTWLGYVNSAVNPIIY$_R$ (SEQ ID NO:55) aa 407-426 of the D2 receptor, within the TM VII domain(Fig. 2B). Both of these peptides, containing no strongly polar residues, had similar ability to dissociate D2 dimers immunoprecipitated from a human caudate preparation as well (Fig. 2C). A small increase in the molecular mass of receptor monomer in samples coincubated with peptide was noted, which may be attributed to the formation of a peptide-D2 receptor heterodimer.

Radioligand ([^{3}H]YM-01951-2) binding to the D2 receptor was completely abolished after treatment of the receptor with the D2 TM VI or TM VII peptides.

Peptide actions were receptor and site-specific since no disruption of D2 dimers was observed under identical experimental conditions using the hydrophilic peptide CTHPEDMKLCTVIMKSNGSF$_N$ (SEQ ID NO:62) aa 244-263 of the D2 receptor C IIIA domain and peptide LSSTSPPERTRYSPIPPSHH$_R$ (SEQ ID NO:63) aa 284-303) of the D2 receptor C IIIB domain or third cytoplasmic loop third cytoplasmic loop of the D2 receptor, or a hydrophobic peptide (aa 276-296) corresponding to a portion of the TM-VI region of the 2-adrenergic receptor (2-AR), or two peptides derived from

the carboxyl tail of the D1 receptor (aa 369-383 and aa 416-431) (Fig. 2D). In addition, no dissociation of immunoprecipitated human dopamine D1 and serotonin 5-HT1B receptor dimers was observed with the D2-TM VII peptide fragment (Fig. 2E). These results indicate that synthetic peptides derived from hydrophobic putative transmembrane domains of receptor proteins can interact specifically to disrupt receptors as noncompetitive antagonists.

In Vivo Receptor Studies

The effect of D2 receptor transmembrane peptides on D2 receptor activity was studied using the accepted animal model described by Costall et al. (1983), for screening dopamine receptor antagonists.

Stereotaxic surgery: Male Wistar rats (~300-350 g) were anaesthetized with ketamine (66 mg/kg i.p.), acepromazine (3 mg/kg i.p.) and pentobarbital (22 mg/kg i.p.) for chronic stereotaxic implantation. A unilateral stainless steel guide cannula (G22) was placed into the centre of the left caudate putamen (Ant. +1.5, Lat. -2.2, Vert. -5.0, Paxinos and Watson, 1982) to allow drug or vehicle injection. Alternatively, bilateral stainless steel guide cannulas (G22) were stereotaxically placed into the centre of left and right caudate putamens for both drug and vehicle injections respectively. The guide cannula was kept patent by stylets (Plastic Products Company, Roanoke, VA) terminating 0.5 mm below the guide tips which were located 2 mm above the point of injection. The rats were allowed a week postoperative recovery before experimental use.

Intracerebral injection technique. The stylets were withdrawn and injections (drug or vehicle) made into conscious rats using a 28 gauge internal cannula connected by PE-50 polyethylene tubing to a gastight

Hamilton syringe. The stylets were then replaced. For unilateral operated animals, a total of 2-3 μl drug in vehicle or vehicle alone was injected into the left striatum, followed 15 min after by a subcutaneous injection of 0.25 mg/kg apomorphine. Rats with bilateral cannulas were administered drug into the left striatum and vehicle into the right striatum, followed 15 min after by a subcutaneous injection of 0.25 mg/kg apomorphine.

Behavioural scoring. The intensity of the behavioral response was assessed every 2-5 min post-injection. Asymmetry (ipsilateral to the side of peptide or vehicle injections) was scored on the 0-3 system, 15 min after subcutaneous challenge with apomorphine (0.25 mg/kg). Animals showing an ability to move in right and left directions were not categorized as circling. However, an ability to circle in one direction (asymmetric body posture) was scored on a 0-3 response according to observations in the open field and to the lifting of the tail. The criteria which met the 0-3 scores were:

0=no asymmetry, response of animals the same as untreated rats.

1=a distinct tendency for animals to move in one direction when handled but still capable of movement in either direction.

2=spontaneous movements in one direction, a twisting of the body in this direction, exaggerated when handled, with inability to move in opposite direction.

3=a marked and intense twisting of the body, active circling movements when disturbed, the animal being unable to move in the opposite direction.

Effect of unilateral intrastriatal injection of receptor peptides on motor behavior. Following unilateral injection of peptides (D2-TM VII, D2-TM II or D2-AR-TM VI) or vehicle into the left striatum, animals were

observed for 1 h. All treatments failed to induce circling responses.

Circling following unilateral intrastriatal injection of D2-TM VII peptide and challenge with subcutaneous apomorphine. Subcutaneous challenge with apomorphine 15 min post unilateral intrastriatal administration of the DS-TM VII, but not other peptides or vehicle, revealed an ipsilateral asymmetric body posturing in rats achieving a behavioral response score of 3 (Fig. 3). Similar ipsilateral asymmetric body posturing in rats was observed in bilaterally cannulated rats following intrastriatal administration of D2-TM VII and vehicle into left and right striatum respectively (Fig. 4). The results indicate that D2-TM VII peptide acts pharmacologically as a classical D2 receptor antagonist.

Example 2

The effect of adrenergic receptor antagonist peptides was examined using an accepted animal model for assessing cardiovascular drugs.

Materials and Methods.

Animals Male Wistar rats (Charles River Breeding Laboratories, St. Constant, QC) weighing about 250-350 gm were utilized. Upon arrival, rats were housed in the animal facility for acclimatisation for 1 week.

Surgery Prior to the experiments, the rats were fasted overnight but provided with water *ad libitum*. Under halothane anesthesia, the left femoral or jugular vein was exposed, cleaned, clamped and cannulated with polyethylene tubing (Tygon; PE 10-20) for intravenous injections of drugs. The right carotid artery was then exposed adjacent to the trachea and cannulated for blood pressure recording (PE 10-20 tubing). The cannulae were tunneled subcutaneously to the midback of the animal where they were brought out onto the skin surface and

capped with rubber injection ports. All catheters were filled with a solution of heparin (10 units/ml) and were flushed periodically with the same solution to prevent clotting.

Cardiovascular measurements Rats were allowed to wake up and recover for 3 hrs prior to cardiovascular measurements. A transducer was connected to the carotid arterial catheter, and blood pressure was recorded on a polygraph. Animals were given atropine 1 mg/Kg subcutaneously 2 to 3 hours before measurement of adrenergic responses to antagonise cholinergic tone.

Measurement of adrenergic receptor responses All drugs were injected intravenously (femoral or jugular vein) in bolus volumes of 0.1 - 0.2 ml and flushed in with 0.2 ml of saline. Blood pressure was recorded continuously by polygraph.

Inhibition of β1-adrenergic receptor activity

After a period of recording baseline cardiac function, in an awake and freely-mobile rat, (480 beats/min, with systolic/diastolic blood pressure of 160/110 min Hg, as seen in Figure 5a), the rat was given 1 μg/Kg of the β1-adrenergic receptor agonist isoproterenol by rapid IV bolus.

This produced a marked increase in spontaneous cardiac activity, with a rise to 600 beats/min and an accompanying reduction in blood pressure to 140/70 (Figure 5b).

After an interval to allow baseline parameters to be reestablished, 100 μl of 5 mg/ml peptide FFNWLGYANSAFNP, a fragment of the β1-adrenergic receptor TM VII peptide, (B1-TM7) was administered intravenously.

As seen in Figure 5c, there was a marked reduction in spontaneous cardiac activity to 240 beats/min with a selective drop in diastolic blood pressure to give 160/60, indicative of β1-adrenergic receptor antagonism.

After baseline parameters were again reestablished, a further 1 mg/Kg isoproterenol was administered. The response to isoproterenol was attenuated, as seen in Figure 5d, showing lasting β1-adrenergic receptor antagonism induced by the TM VII peptide fragment

Figures 5e to 5h show the results of a control experiment. Baseline cardiac parameters were 240 beats/min and blood pressure 130-125/80 (Fig. 5e). Fig. 5f shows the response to 1 mg/Kg isoproterenol, resulting in reduced baseline blood pressure 125/60 characteristic of β1-adrenergic receptor activation. Administration of peptide vehicle (Buffer A + 10% DMSO) slightly reduced blood pressure to 140/50 but did not change spontaneous cardiac activity (Fig. 5g).

Figures 6e to 6g show the results of a control experiment in the same rat. Administration of saline resulted in an unexpected transient increase in blood pressure from 150/100 to 180/100, with no change in heart rate, before stabilising to baseline values (Figure 6e).

Administration of peptide vehicle had no significant effect on heart rate, but lowered blood pressure marginally from 150/100 to 140/80 (Fig. 6g). Subsequent administration of 5 μg/Kg phenylephrine showed no diminution of effect and reduced heart rate to 240 beats/min with blood pressure rising to 180/140 (Fig. 6f).

After baseline parameters were reestablished, exposure of the animal to 1 mg/Kg isoproterenol showed characteristic heart beat and blood pressure changes (Fig. 5h) with no attenuation of the drug's initial effect.

Inhibition of α1A-adrenergic receptor activity

After a period of recording baseline cardiac function in an awake and freely-mobile rat (360 beats/min

with systolic/diastolic blood pressure of 130/90, as seen in Figure 6a), the rat was given 5 μg/Kg phenylephrine by rapid IV bolus.

Heart rate was reduced to 240 beats/min, with blood pressure rising to 180/140, reflecting a drug-induced vasoconstriction effect (Fig. 6b).

After an interval to allow baseline parameters to be reestablished, 100 μl of 5 mg/ml peptide VFKVIFWLGYFNSCVN, a fragment of the α1A-adrenergic receptor TM VII peptide (alpha tm7) was administered intravenously.

As seen in Figure 6c, heart rate was reduced to 240 beats/min before recovery to 420 beats/min, during which time there was a significant, transient drop in blood pressure to 130-120/60.

After baseline parameters were again reestablished, a further 5 μg/Kg phenylephrine was administered. The response to phenylephrine was delayed and attenuated indicating lasting α1A-adrenergic receptor antagonism induced by the TM VII peptide fragment (Figure 6d).

Effect of prasozin

As a comparison, the effect of prasozin, a classical α1A-adrenergic receptor antagonist, was examined (Figures 7a to 7d).

As seen in Figure 7, prasozin (1 mg/Kg by rapid IV bolus) had an effect on heart rate and blood pressure (Figure 7c) similar to that of the antagonist TM VII peptide fragment and also caused attenuation of the response to a subsequent administration of the agonist phenylephrine (Fig. 7d).

Example 3

Saturation experiments on D1 and D2 dopamine receptors were carried out by membrane radioligand binding assay.

Briefly, for the D1 receptor, saturation experiments were done with increasing concentrations of antagonist [^{3}H]SCH-23390 (10-4000 pM, final concentration), whereas for the D2 receptor, saturation experiments were done with increasing concentrations of antagonist [^{3}H]spiperone (10-2000 pM, final concentration). Tubes were incubated for 90 minutes at 22°C in a final volume of 1 mL with binding buffer C (50 mM Tris HCl; 5 mM EDTA; 1.5 mM $CaCl_2$; 5mM $MgCl_2$; 5mM KCl) with NaCl for antagonists, without NaCl and with ascorbic acid for agonists. For saturation and competition experiments, nonspecific binding was defined as binding that was not displaced by 1 μM (+)butaclamol and membrane protein concentrations of ~25 μg per tube were used. Bound ligand was isolated by rapid filtration through a Brandel 48-well cell harvester using Whatman GF/C filters. Filters were washed with 10 mL of cold 50 mM Tris-HCl buffer (pH 7.4) and counted for tritium.

The present invention is not limited to the features of the embodiments described herein, but includes all variations and modifications within the scope of the claims.

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TABLE 1 - G-PROTEIN COUPLED RECEPTORS

adenosine receptors	antidiuretic hormone receptor
Alpha-adrenoceptors	angiotensin receptors
bradykinin receptors	beta-adrenoceptors
Blue Opsin receptor	bombesin receptor
Calcitonin receptor	cannabinoid receptors
dopamine receptors	endothelin receptors
follicle stimulating hormone receptor	gastrin receptor
Green Opsin receptor	histamine receptors
interleukin-8 receptor	luteinizing/chorionic gonadotropin receptor
muscarinic receptors	neuromedin K
neuropeptide Y	odorant receptors
opiate receptors	opsin receptors
oxytocin receptor	peptide YY receptor
prostaglandin E	parathyroid hormone receptor
RDC1	Red Opsin receptor
Rhodopsin receptor	serotonin receptors
somatostatin receptors	substance K
substance P	thrombin receptors
thromboxane A2 receptors	thyrotropin-releasing hormone receptors
thyroid stimulating hormone receptor	tyramine receptors
vasopressin V1a receptor	vasoactive intestinal peptide receptor

TABLE 2

DOPAMINE D2 RECEPTOR, Human

443 Amino acids

ACCESSION: P14416

FEATURES

DOMAIN	1	37	EXTRACELLULAR (POTENTIAL).
TRANSMEM	38	60	1 (POTENTIAL).
DOMAIN	61	71	CYTOPLASMIC (POTENTIAL).
TRANSMEM	72	97	2 (POTENTIAL).
DOMAIN	98	108	EXTRACELLULAR (POTENTIAL).
TRANSMEM	109	130	3 (POTENTIAL).
DOMAIN	131	151	CYTOPLASMIC (POTENTIAL).
TRANSMEM	152	174	4 (POTENTIAL).
DOMAIN	175	186	EXTRACELLULAR (POTENTIAL).
TRANSMEM	187	210	5 (POTENTIAL).
DOMAIN	211	373	CYTOPLASMIC (POTENTIAL).
TRANSMEM	374	397	6 (POTENTIAL).
DOMAIN	398	405	EXTRACELLULAR (POTENTIAL).
TRANSMEM	406	429	7 (POTENTIAL).
DOMAIN	430	443	CYTOPLASMIC (POTENTIAL).

SEQUENCE

MDPLNLSWYD DDLERQNWSR PFNGSDGKAD RPHYNYYATL LTLLIAVIVF GNVLVCMAVS
REKALQTTTN YLIVSLAVAD LLVATLVMPW VVYLEVVGEW KFSRIHCDIF VTLDVMMCTA
SILNLCAISI DRYTAVAMPM LYNTRYSSKR RVTVMISIVW VLSFTISCPL LFGLNNADQN
ECIIANPAFV VYSSIVSFYV PFIVTLLVYI KIYIVLRRRR KRVNTKRSSR AFRAHLRAPL
KGNCTHPEDM KLCTVIMKSN GSFPVNRRRV EAARRAQELE MEMLSSTSPP ERTRYSPIPP
SHHQLTLPDP SHHGLHSTPD SPAKPEKNGH AKDHPKIAKI FEIQTMPNGK TRTSLKTMSR
RKLSQQKEKK ATQMLAIVLG VFIICWLPFF ITHILNIHCD CNIPPVLYSA FTWLGYVNSA
VNPIIYTTFN IEFRKAFLKI LHC

TABLE 3

INTEGRAL MEMBRANE PROTEIN	SwissProt ACCESSION NO.
Adenosine A1 Receptor	P30542
Adenosine A2a Receptor	P29274
Adenosine A2b Receptor	P29275
Adenosine A3 Receptor	P33765
Adrenoceptor α1A	P25100
Adrenoceptor β1	P08588
Adrenoceptor β2	P07550
Angiotensin II Receptor-Type 1A	P30556
Bombesin Receptor, (for Gastrin-Releasing Peptide)	P30550
Bombesin receptor for Neuromedin-B	P28336
Bombesin Receptor Subtype-3	P32247
Bradykinin-1 Receptor	P46663
Bradykinin-2 Receptor	P30411
Calcitonin Receptor	P30988
Calcium-Sensing Receptor precursor	P41180
Cholecystokinin Type A Receptor	P32238
Dopamine D1 Receptor	P21728
Dopamine D2 Receptor	P14416
Dopamine D3 Receptor	P35462
Dopamine D4 Receptor	P21917
Dopamine D5 Receptor	P21918
Endothelin Receptor	P25101
Endothelin B Receptor	P24530
Epidermal Growth Factor	P00533; P06268
Follicle Stimulating Hormone Receptor	P23945
γ-Aminobutyric-Acid Receptor α-1 Subunit	P14867
γ-Aminobutyric-Acid Receptor α-2 Subunit	P47869
γ-Aminobutyric-Acid Receptor α-3 Subunit	P34903
γ-Aminobutyric-Acid Receptor α-4 Subunit	P48169
γ-Aminobutyric-Acid Receptor α-5 Subunit	P31644

TABLE 3 continued

γ-Aminobutyric-Acid Receptor β-1 Subunit	P18505
γ-Aminobutyric-Acid Receptor β-2 Subunit	P47870
γ-Aminobutyric-Acid Receptor β-3 Subunit	P28472
γ-Aminobutyric-Acid Receptor γ-2 Subunit	P18507
γ-Aminobutyric-Acid Receptor ρ-1 Subunit	P24046
γ-Aminobutyric-Acid Receptor ρ-2 Subunit	P28476
Gastrin/Cholecystokinin Type B Receptor	P32239
Gonadotropin-Releasing Hormone Receptor	P30968
Histamine H1 Receptor	P35367
Histamine H2 Receptor	P25021
Interleukin-1 Receptor, Type I	P14778
Interleukin-1 Receptor, Type II	P27930
Interleukin-2 Receptor β Chain	P14784
Interleukin-2 Receptor γ Chain	P31785
Interleukin-3 Receptor α Chain Precursor	P26951
Interleukin-4 Receptor α Chain	P24394
Interleukin-5 Receptor α Chain	Q01344
Interleukin-8 Receptor B	P25025
Low-Density Lipoprotein Receptor	P01130
Muscarinic Acetylcholine M1 Receptor	P11229
Muscarinic Acetylcholine M2 Receptor	P08172
Muscarinic Acetylcholine M3 Receptor	P20309
Muscarinic Acetylcholine M4 Receptor	P08173
Muscarinic Acetylcholine M5 Receptor	P08912
Neuromedin K or Neurokinin B Receptor	P29371
Neuropeptide Y Receptor, Type 1	P25929
Neuropeptide Y Receptor, Type 2	P49146
MU-Type Opioid Receptor, MOR-1	P35372
Oxytocin Receptor	P30559
Parathyroid Hormone Receptor	P49190
Serotonin 1a Receptor, 5-HT_{1a}	P08908

TABLE 3 continued

Serotonin 1b Receptor, 5-HT_{1b}, or 5-$HT_{1d\beta}$	P28222
Serotonin 1D Receptor, 5-HT_{1d}, or 5-$HT_{1d\alpha}$	P28221
Serotonin 1e Receptor, 5-HT_{1e}	P28566
Serotonin 1F Receptor, 5-HT_{1F}	P30939
Serotonin 2A Receptor, 5-HT_{2A}	P28223
Serotonin 2B Receptor, 5-HT_{2B}	P41595
Serotonin 2C Receptor, 5-HT_{2C}	P28335
Serotonin 5a Receptor, 5-HT_{5a}	P47898
Serotonin 7 Receptor 5-HT_{7}	P34969
Somatostatin Receptor Type 1	P30872
Somatostatin Receptor Type 4	P31391
Somatostatin Receptor Type 5	P35346; P34988
Sustance K Receptor or Neurokinin A	P21452
Substance P or Neurokinin 1 Receptor	P25103
Thrombin Receptor Precursor	P25116
Thromboxane A2 Receptor	P21731
Thyroid Stimulating Hormone Receptor	P16473
Thyrotropin-Releasing Hormone Receptor	P34981
Vasoactive Intestinal Polypeptide Receptor 1	P32241
Vasopressin V1A Receptor	P37288
Vasopressin V1B Receptor	P47901
Vasopressin V2 Receptor	P30518
Sodium- and Chloride-Dependent Betaine Transporter (NA+/CL-Betaine/GABA Transporter) (BGT-1)	P48065
Dopamine Transporter	Q01959
Excitatory Amino Acid Transporter 4 (Chloride-Dependent Glutamate/Aspartate Transporter)	P48664
Excitatory Amino Acid Transporter 1 (Sodium-Dependent Glutamate/Aspartate Transporter 1) (Glial Glutamate Transporter)	P43003
Norepinephrine Transporter	P23975
Serotonin Transporter	P31645